Sediment-Water Interface Exposure System

1.0 OBJECTIVE

The purpose of the Sediment-Water Interface (SWI) test is to assess toxicity of solid phase sediment samples using the embryo/larval stages of marine and estuarine invertebrates and vertebrates. Some freshwater species have also been used successfully in the SWI exposure system. In this procedure, sediment samples are placed into test chambers that are then filled with uncontaminated overlying seawater. Screen tubes are then placed into the test chambers so that the screen is almost in contact with the sediment. After a 24-h equilibration period, test organisms are inoculated into the screen tubes, where they develop and live in proximity to the sediment. The size of the screen is small enough to retain the organisms but large enough to allow for passive diffusion of chemicals into the screen tube. At the termination of the test the screen tube is removed and the organisms are washed into a separate container for microscopic evaluation. In this test system most of the animals are retained, allowing for accurate quantification of survival and developmental abnormalities (Anderson et al. 1996).

At MPSL this sediment exposure method has been developed for marine and estuarine applications using the sea urchin or mussel embryo development protocols, but has also been used with the freshwater cladoceran *Ceriodaphnia dubia*. This exposure system may be used to assess the toxicity of intact (undisturbed) sediment core samples, thereby eliminating artifacts that result from the manipulation of sediment and pore water samples. The exposure system may be combined with analyses of toxicant flux measures to provide biological information on the effects of contaminated sediments on the water column.

2.0 EQUIPMENT

The following equipment is necessary to conduct the toxicity test at the Marine Pollution Studies Laboratory at Granite Canyon (MPSL). The word "clean" here and throughout this procedure means that the item has been cleaned according to the MPSL glassware cleaning procedures outlined in a separate standard operating procedure (MPSL SOP 1.3).

2.1 Organism Collection and Culture

• Tanks, trays, or aquaria for holding organisms, e.g. standard seawater aquarium with appropriate filtration and aeration system.
• Air pump, airlines, and air stones -- for aerating water containing adult urchins (for static systems and emergency aeration for flow-through systems).

2.2 Test Initiation

• Polycarbonate tubing for sediment cores (7.5 cm ID)
• Polyethylene plastic caps for cores (7.5 cm)
• Polycarbonate tubing for exposure screen tubes (e.g., 5 cm ID)
• Plastic Cement for screen construction

• Polyethylene screen
• Parafilm® for sealing cores
• Beakers, 1,000 mL borosilicate glass
• Wash bottles - for dilution water and distilled water.
• Constant temperature chambers or water baths.
• Pipettes, automatic - adjustable, to cover a range of delivery volumes from 10 to 1000 µL.
• Hemacytometer
• Sedgwicke-Rafter counting cell
• Mixing Plunger (for mixing gametes)
• Graduated cylinders - Class A, borosilicate glass or non-toxic plastic lab ware.
• Tape, colored - for labeling tubes and other containers.
• Polypropylene Spoons
• Markers, waterproof - for marking containers, etc.
• Gloves, disposable - for personal protection from contamination.

2.3 Test Termination

• Inverted and compound microscope for inspecting gametes and making counts of embryos and larvae.
• Data sheets.
• Formaldehyde, 37% (Concentrated Formalin) for preserving embryos and larvae.
• Fume hood to protect the analyst from effluent or formaldehyde fume
• Counter, two unit, for recording counts of embryos and larvae.

2.4 Water Quality

• Meters and probes for measuring pH, dissolved oxygen, and ammonia
• Refractometer for measuring salinity
• Thermometers (glass mercury thermometer and continuously recording chart thermometer)
• Graduated pipettes (10 mL) and hand pipette pump for water quality sampling
• Gloves and appropriate safety gear (see MPSL lab safety manual)

2.5 Dilution Water

For tests at Marine Pollution Studies Laboratory, dilution water is ambient Granite Canyon seawater, filtered to 1 µm, at ambient salinity (33-34‰). This water is used to prepare eggs and sperm for toxicity tests, and for diluting test solutions.

3.0 EXPERIMENTAL DESIGN AND PREPARATION

3.1 Sampling Procedures

The following procedures are divided into sections for testing homogenized sediment and intact (non-homogenized) surficial sediment samples. The SWI method has been used primarily for testing marine and estuarine sediment samples and the screen tubes described here were designed for exposing invertebrate and vertebrate embryos larger than 25 µm in diameter.

3.2 Screen Tube Construction

Screen tubes are constructed from clear, polycarbonate stock. Screen tube size will very with species and protocols; for using the sea urchin embryo/larval protocol screen tubes are constructed from 4 cm (ID) diameter stock that is cut into 15 cm high sections on a conventional band saw. The wall thickness is 3 mm. A 1-cm section is cut from the bottom of the tube and this serves as the pedestal that sits on the sediment surface. Polyethylene (PECAP®) screen is glued to the tube using clear-thickened acrylic plastic glue and the pedestal is then glued back on the tube to sandwich the screen. A small hole is drilled into the side of the pedestal that is used to purge any air trapped under the screen during immersion. Screen size will vary depending upon the application. Twenty-five micron screen is appropriate for the sea urchin and mussel embryo development protocol. Polyethylene mesh is stronger than conventional nylon mesh and better withstands repeated solvent rinses.

Polycarbonate core tubes are used for collecting intact, un-homogenized samples (see below). Cores are constructed by cutting 7.3-cm (ID) stock into 20-cm high sections. Polyethylene caps are used to seal samples inside the tubes.

3.3 Homogenized Sample Handling

The following procedures are for conducting solid-phase SWI tests using the embryo development protocols. Sediment collection and processing procedures follow guidelines described in ASTM (1993). It is assumed that test sediments are homogenized according to this method prior to loading into the test containers. Sediment and overlying water are added to the cores 24-hour prior to inoculation of the test organisms to allow samples to equilibrate.

Prior to loading homogenized sediment into the cores, polyethylene caps are placed on one end of each core and this end is sealed with Parafilm®. After labeling, the cores are arranged in groups of 5 replicates per sample with one extra core per sample to be used for overlying water quality measurements at the termination of the test.

Using a polypropylene spoon, sediment is placed into each tube forming a layer 5-cm deep. Lower a clean plastic disc (attached to a pipette) into the tube approximately 1 cm above the surface of the sediment. Add 300 mL of "clean" overlying seawater (ambient salinity, 15°C) so as not to disturb the sediment. Slowly lift the disc out as the

water is added. The cores are arranged in a constant temperature room and covered with acrylic sheets containing glass pipettes that deliver gentle aeration (1 bubble/second). The sediment and overlying water are allowed to equilibrate overnight before introduction of the screens and test organisms. A screen tube is added to each core container the following morning. The screen tube is gently placed in the core so that the bottom collar rests on the substrate, this leaves the screen itself 1 cm above the substrate. The water in the outer core tube should be 12 cm deep, resulting in 150 mL of water in the screen tube itself.

3.4 Intact Sediment Sample Handling

Methods for handling intact core samples are essentially the same as those described above, with the following modifications designed to minimize disturbance of the sample.

The core may be taken directly from the sampling device, by hand from an intertidal sampling site or, sub-tidally using divers, directly from the sediment surface. Depth of the sample will depend on the study goals. At MPSL, we generally take 5 cm deep cores because this is the practical sampling depth of the modified Van Veen grab sampler used at our laboratory. The core is pressed into the sediment and a pre-cleaned acrylic plate or a gloved hand is inserted under the bottom of the core to prevent leakage of sample or interstitial water as the sample is removed. It is convenient to mark the 5-cm height for reference using a plastic cable tie wrapped around the outside of the core. After the core is removed from the sediment, the bottom is capped quickly; then the top is capped. A small hole in the top cap relieves positive pressure on the sample and minimizes leakage as the cap is attached. Sample integrity is verified by the presence of sediment overlying water. If an inordinate volume of interstitial water or sediment leaks out, the sample is discarded and a new one collected. The outside of the tube is dried and the bottom wrapped tightly in Parafilm® to prevent leakage. The core is then stored upright and iced.

Approximately 24 hours prior to initiation of the toxicity test, the overlying water in the core (from the sample site) is gently siphoned off of the top of the sample leaving about 0.5 cm of water remaining to minimize disturbance of the sediment surface microlayer. Three hundred milliliters of "clean" overlying seawater (ambient salinity, 15°C) is then introduced into the cores using acrylic disks to minimize sample disturbance, as described above. Unlike homogenized samples, salinity of interstitial water may not be adjusted in the intact samples. As described above for homogenized samples, the cores are usually arranged in groups of 5 replicates per sample with one extra core per sample to be used for interstitial sulfide and ammonia measurements at the termination of the test. Screen tubes are gently added to the cores at the beginning of the day of the test. The test is run under ambient laboratory lighting conditions.

4.0 TOXICITY TESTING PROCEDURES

The following procedures are patterned after US EPA 1995. The purpose of the embryo/larval development tests are to determine if sediment samples cause abnormal development of exposed embryos relative to embryos exposed

to control or reference samples. For details, refer to MPSL Standard Operating Procedures for the appropriate test organism.

5.0 REFERENCES

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